

The University of Chicago

SPLEEN STUDIES

I. MICROSCOPIC OBSERVATIONS OF THE CIRCULATORY SYSTEM OF LIVING UNSTIMULATED MAMMALIAN SPLEENS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1935

By
MELVIN HENRY KNISELY

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EIGHT FIGURES

INTRODUCTION

Repeated study of the problem of splenic circulation by the classical histologic procedures of injection, fixation, and sectioning has failed to elucidate either the structure of the splenic vascular system or the course of the blood through it. So I have studied living transilluminated mammalian spleens microscopically in an attempt to learn how blood circulates through this organ.

Study of some fixed spleens has led to the development of the concept of a 'closed' circulation. This conception is, that blood is always inside of preformed, interconnected, lined channels. Study of other fixed spleens has yielded the concept of the 'open' circulation, which postulates that the arterioles, or capillaries, pour blood freely into the intercellular spaces of the splenic pulp tissue, and from these spaces the blood finds its way into the veins again. There are a variety of modifications of these two antagonistic concepts. The modifications of the concept of the 'open' circulation are especially numerous. The modifications of each of the two views are based

¹ This work was aided by a grant from the Rockefeller Foundation to the Biological Sciences Division of The University of Chicago.

The studies of living transilluminated organs are being made under the direction of Dr. R. R. Bensley. Dr. William Bloom suggested that the spleen be studied by this method. The assistance and counsel of Doctor Bensley, Doctor Bloom and the other members of the staff of Hull Anatomical Laboratory have been invaluable in this work.

largely upon different results which have been obtained in attempting to deduce the course of the blood through the spleen by observing the post mortem position of various injected foreign materials.

One point of controversy relates to the length and ultimate connections of the splenic arterioles, arterial capillaries and capillaries. Some investigators can trace lined arterioles only as far as the ellipsoids of Schweigger-Seidel, while others find that after passing through the ellipsoids, the arterial vessels divide, some branches connecting directly with venous sinuses others penetrating the pulp 'cords' for various distances. MacNeal ('29) traced these 'long, curved' capillaries a long way into the pulp 'cords.' Why is it that these arterial capillaries can be traced a long way by some, only a short distance by others, and cannot be traced past the ellipsoids by other investigators? Why cannot everyone find the connections of arterial capillaries with venous sinuses?

Another difficult point is the presence of a variable number of erythrocytes in the intercellular spaces of the pulp 'cords' in fixed spleens. If the vascular system is 'closed,' then how do erythrocytes get into these intercellular spaces? On the other hand, if the system is 'open,' why is it that there is not as great a concentration of red cells in the pulp 'cords' as there is in hemorrhagic tissue?

A number of investigators have injected colloidal sols into the splenic arteries and studied the position of the injected colloid in the splenic pulp. Sections of spleens so prepared almost always show the colloidal material in the intercellular spaces of the pulp 'cords.' How can this kind of result be explained in terms of the 'closed' splenic vascular system?

It has been postulated by some investigators that a 'closed' circulation in a contracted spleen may become an 'open' circulation when the organ is dilated. And it has been suggested that physiological variations—other than the degree of dilatation of the organ—might account for the structural variations reported.

This is the first of a series of papers dealing with the structure and activities of the splenic vascular system. In this first paper the unstimulated spleen will be described; that is, the structure and activities which are observed while the spleen and its attachments are as carefully protected from external manipulative stimulation as possible.

Another paper will describe, 1) the reactions of the splenic circulatory system to the stimulation of experimental manipulation and trauma, and 2) the changes which rapidly take place in the spleen during the brief period while the animal is dying.

Subsequent papers will probably take up the reaction of the spleen to injected foreign materials and a review of the literature which relates to some of the problems of the splenic vascular system.

MATERIALS AND METHOD.

The spleens of seventy-five white mice, thirty white rats and fifteen cats were studied. The cats were all between 3 and 12 weeks old. Mice and rats of all ages, from nurslings to adults were used, in order to see if there were age variations in the splenic vascular system. Because it had been postulated that dilated spleens have an 'open' circulation and contracted spleens a 'closed' circulation, spleens have been studied in many different degrees of dilatation. The spleen is very large during and immediately after the ingestion of food, and progressively decreases in size thereafter. So, to study dilated spleens, animals were taken from the feeding trays and observations of the spleen begun immediately. Other spleens were studied, beginning after various intervals had elapsed since the animal's last meal; the elapsed time intervals varied from a few minutes up to and including overnight, 14-hour fasts.

Because the spleen contracts when an animal is exercised or in some emotional states (Barcroft and Stephens, '27), it was necessary to be sure that the animals used were not in an exercised or frightened condition. For that reason most of

the animals were accustomed to being freely handled and to living in the laboratory before they were used in this study.

The spleens of frightened and exercised animals were also observed. To frighten and exercise an animal it was placed in a pail and frightened into running by noisily rapping the pail with a stick.

Sodium amytal, injected subcutaneously at the back of the neck or over the buttocks was the anesthetic. If a fine sharp needle is used, sodium amytal solution can be quickly injected without frightening a tame animal enough so that its spleen contracts. In the longer observations the animals have been given small extra doses of sodium amytal from time to time to maintain that concentration in the animal necessary for light anesthesia.

The position of the spleen is determined by very gentle palpation, and an incision then made in the abdominal wall, just posterior to the ribs in such a position that the incision just crosses the area under which the spleen lies. The incision is made between small hemostats to prevent blood loss. (The hair has been clipped off this area a day or two earlier.) The slit in the body wall is now pulled posteriorward to the posterior-inferior end of the spleen and the posterior lip of the incision depressed until it passes under the end of the spleen. As the tissue of the body wall shortens again the spleen remains in its usual position except that its posterior-inferior end is now outside the body. During this manipulation the spleen itself is not touched with fingers or instruments. (The only instrument ever used to touch the spleen is a smooth, paraffin-coated, wooden probe.) All tension and torsion of the spleen and its mesentery (gastrosplenic ligament) are carefully avoided. The best results are obtained in cases in which only a small portion of the ventral end of the spleen is exposed through a hole which is almost filled by the protruding spleen. Precautions are taken in each case to insure that the incision is large enough so that the blood vessels to the spleen are not compressed and that the incision is no larger than is necessary (fig. 1). The spleens

were transilluminated by means of the fused quartz rod previously described (Knisely, '36). The vascular system of the spleen is more sensitive to temperature variations than is the vascular system of most other organs, so it is necessary to take strict precautions to maintain the temperature of the exposed portion of the spleen at the temperature of the abdominal cavity of the animal. One cannot use metallic instruments to touch the spleen because of the rapidity with which metal at room temperature conducts heat from tissue

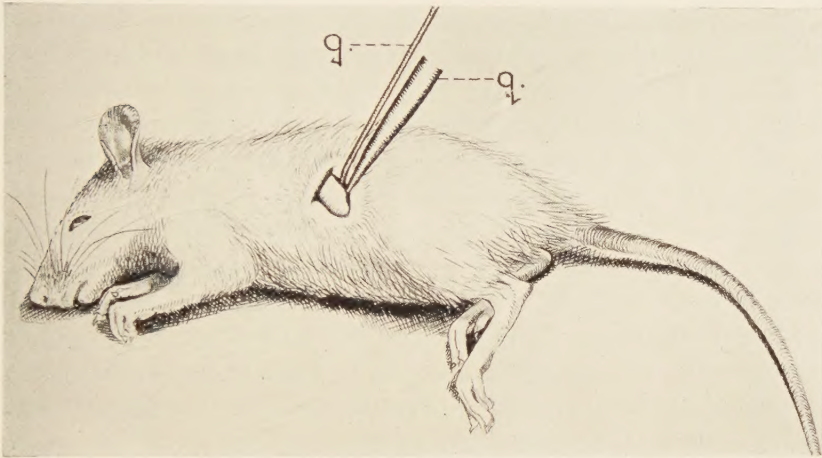


Fig.1 Young rat with posterior-ventral end of spleen exposed for study. Quartz rod (q) and glass tube (g) in position to illuminate a small area of the spleen.

which is at mammalian body temperature. Also metallic instruments in the Ringer's solution act as electrodes, so developing electrical potentials which sometimes bring about vasomotor reactions.

The structures of the circulatory system were studied by focusing into the thinner portions (medial and lateral surfaces of the sides and ends) of the organ. One can be certain of studying structures well below the surface of the organ by focusing first on the surface, noting the fine adjustment reading, then focusing deep and noting the fine adjustment reading again. A low power binocular dissecting

microscope and monocular and binocular microscopes were used in the work. The magnifications secured with the latter varied from $\times 60$ to $\times 600$. The higher magnifications were secured by using a long focus $\times 40$ water immersion objective lens in combination with $\times 10$ or $\times 15$ oculars. Most of the observations were made or checked with the $\times 40$ water immersion objective and $\times 10$ oculars.

The majority of the observations were made using the whole visible spectrum. However, a green filter aided in differentiation in many cases.

The preliminary observations showed that the tissue of the spleen is more sensitive and more reactive than had previously been suspected. So, in attempting to observe the routine activities of the splenic vascular system (rather than its reactions to the stimuli produced by the manipulations of experimental procedure) it was necessary to arrange the experiment so that the animal could be undisturbed for as long a time as possible. When an area was found that was especially favorable for study, the illuminating apparatus was adjusted, and from that time on the spleen and the whole animal were carefully left undisturbed while the particularly favorable area was watched continuously until some change (usually in the position of the spleen) took place, which necessitated a readjustment of the apparatus. The microscope could be freely moved from field to field, and the temperature of the wash solutions could be read and adjusted without disturbing the spleen or the animal.

OBSERVATIONS ON UNSTIMULATED SPLEENS

I. The general appearance of living splenic tissue

The capsule and trabeculae are practically transparent when brightly illuminated. The positions of larger trabeculae can be determined by noting the position of the larger veins and the position of the small pits where trabeculae exert tension on the capsule. The pulp tissue is transparent or translucent, depending upon the intensity of illumination used.

The malpighian bodies are irregularly ovoid. Near their surfaces they are translucent but they are more opaque toward their centers. I have not yet been able to observe an exact limiting surface between them and the surrounding tissue. The structures of the living splenic vascular system stand out like the injected vascular systems of 'whole mount' preparations of other organs. So, as in studying a whole mount, one has to focus up and down, and move along the vessels to trace out their connections.

The sheaths of Schweigger-Seidel are also transparent but their position can be determined because they are at or immediately beyond the penicilli tips, and the penicilli are easily recognized.

The venous sinuses are seen to be definite anatomical units, shaped roughly like sweet potatoes, or like cucumbers. They penetrate the tissue in all directions, each like a short, narrow-ended tunnel drilled in a cheese. Their connections to arterial and venous systems, interconnections, and activities are described below. The shape and size of a sinus varies, depending upon which phase of its physiological activity it is in. Venous sinuses are specific anatomical units and are not to be confused, either structurally or functionally, with capillaries, venules or arterio-venous anastomoses.

The tissue between the sinuses consist of thick or thin partitions; the thickness of a given partition at a given time depends upon how much it is squeezed between, and stretched by, the adjacent venous sinuses. The increase in size of the sinuses during some phases of their activity concomitantly decreases the thickness of the partitions, and vice versa. In cutting sections of the spleen this set of irregular partitions is cut in various directions thus creating, by the act of cutting, the appearance of irregular rods and bars known as 'Billroth cords.' The splenic pulp of mice, rats and cats does not consist of a three-dimensional network of cords suspended in blood.

There are relatively few red cells in the pulp partitions (that is, 'Billroth cords')² of the living unstimulated spleen.

² The term 'pulp partitions' will be used from here on, rather than the terms 'Billroth cords' or 'pulp cords.'

In the experiments in which the greatest care has been taken in preparing the spleen for observation the number of red cells in the pulp partition tissue is very small. Each of the few red cells seen in the pulp partitions of an unstimulated spleen travels slowly across the partition. Each travels by itself. No two follow each other as if they were in a common channel. An erythrocyte does not travel by 'fits and starts' as though it were being washed along, and catching and breaking away from the corners of an irregular patent labyrinth made up of interconnected patent intercellular spaces. The rate of red cell movement across the pulp partitions is nearly uniform and quite slow, as though they were traversing very narrow or potential spaces. As a red cell moves along it may change shape somewhat, but usually its shape does not alter greatly.

When a red cell passes through the lining of the vascular system its shape is distorted in a characteristic manner as it begins to penetrate the lining, and the red cell 'snaps' forward suddenly, as soon as the major part of its protoplasm has passed through the lining membrane. (This snapping forward is like the snapping forward of a moist apple seed when pinched between one's thumb and forefinger.) The initial distortion of the red cell I take to be evidence that the lining membrane offers resistance to the penetration of the erythrocytes and the snapping forward to be evidence that the lining membrane is elastic and exerts pressure on the erythrocyte which is traversing it.

This characteristic 'distortion and snap' has been used as one of the criteria in determining whether the membrane lining a blood vessel was continuous or had gaps in it. Each time that an erythrocyte has been seen to pass through a visible blood vessel lining it has been seen to 'distort and snap.' A red cell does not behave in this way when traversing pulp partition tissue, nor when passing through a visible gap in a visible blood vessel lining.

The lining of arterioles, arterial-capillaries, capillaries, venous sinuses and venules appears in the living spleen as a narrow, clear, sharply refractile line. This line is visibly distinct from, and cannot be confused with, the peripheral plasma

layer of the flowing blood stream, for one can see the peripheral plasma layer and the lining of the vessel, a line marking their mutual surface, and one can sometimes see leucocytes rolling along the internal surface of the vessel lining. Pairs of these refractile lines mark the position of the vessel both during the flow of blood and during periods in which there is no blood in the vessel. The refractile linings of the vascular system of the living unstimulated spleen are as continuous as, but thinner than, the refractile linings of arterioles, capillaries and venules in most other tissues, such as smooth muscle, for instance. Each vessel traced was attached to other vessels at each of its ends.

II. Observations on the anatomical relationships of the vascular system of the living spleen

The artery of the malpighian body divides into three or four (occasionally as many as seven) branches, each of which subdivides into from two to four very straight secondary branches, which are penicilli. From each of the penicilli from two to four short branches are given off, the subdivisions of which divide into arterial capillaries.³

The branches of the penicilli are connected with each other by a few lateral anastomoses of capillary diameter. These anastomoses are not limited to interconnecting the branches of a single penicillus, but interconnect branches of adjacent penicilli as well.

The sides of the malpighian bodies are sufficiently transparent so that the outer capillaries of the body can be traced a considerable distance inward. The capillaries of the malpighian body form an anastomosing three-dimensional network. (The term 'loops' which was applied to this network by Nisimaru and Steggerda ('32) is misleading, for the term implies that this capillary network has no efferent connections.) The pattern of this network is not essentially different

³ This is the general pattern of the branching of the splenic arterial system. In the spleen, as in other parts of the mammalian vascular system, there are variations from the general pattern of branching.

from that of the capillary system of several other tissues. The capillaries of the malpighian body (which are frequently said to end by opening out into and connecting with intercellular pulp spaces) peripherally join the capillary network which interconnects the branches of the penicilli (fig. 2).

The branches of the penicilli divide into two or three or four arterial capillaries.

There are two anatomically different types of preformed, lined, vessel systems in the red pulp which connect arterioles or arterial-capillaries to venules. One type consists of venous sinuses and venous sinus systems. The other consists of long, straight or somewhat curved, capillaries. No anastomoses between these two types have yet been found.

Many of the arterial capillaries after a short course connect directly with the afferent ends of venous sinuses. A smaller number enter laterally. There are sinuses which have no other type of afferent, and connect directly with a venule (as at sinus *a* in fig. 2). This type of connection constitutes a single sinus route. There are also multiple sinus routes. Thus the efferent end of a venous sinus may be connected to the end or side of a second sinus as at *b* and *c* in figure 2. The multiple sinus routes have two or three or more consecutive venous sinuses interposed between arterial capillary and venule. (Compare sinuses I, II, III and III *a* of fig. 2, which constitute a multiple sinus route.) There is a functional as well as a structural difference which distinguishes single from multiple sinus routes.

Each single and each multiple sinus route is a definite anatomical unit. The anatomical connections of a given route have remained constant throughout all observed physiological cycles of that route.

For consideration of fluid flow, it is important to note that, though some sinuses have side afferents and some do not, each sinus has an end afferent.

Many arterial capillaries derived from the subdivisions of the penicilli pursue a long, somewhat curved, unbranched

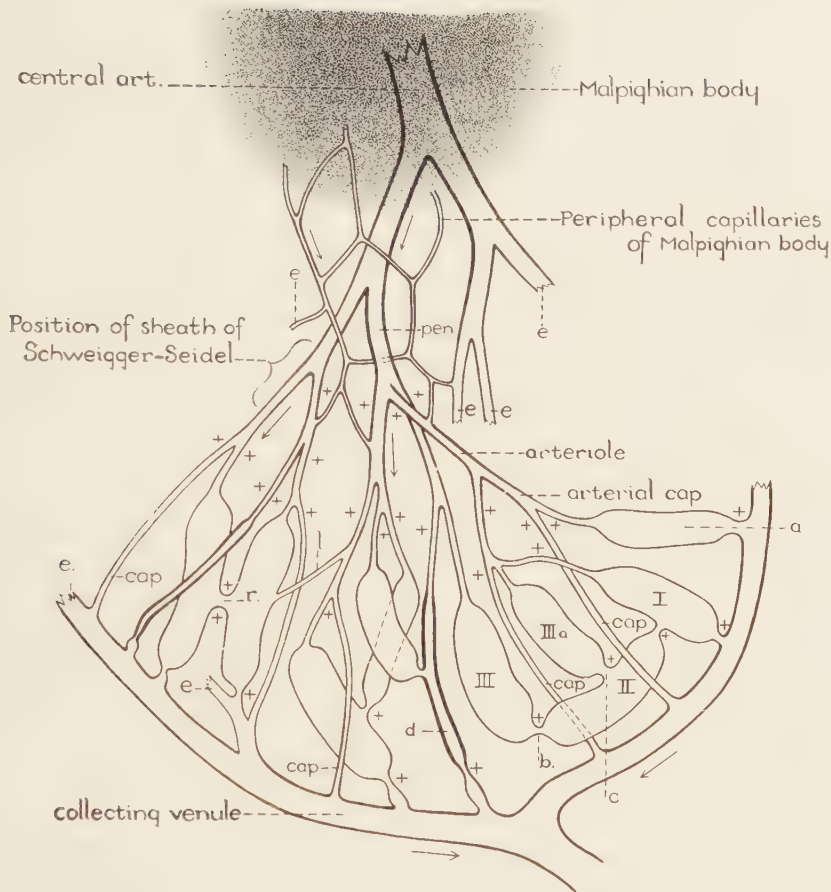


Fig. 2 Diagram of the types of vascular connections which have thus far been seen in the spleen.

I, II, III and *III a* are the individual venous sinuses making up a multiple sinus system. All four are shown in storage phase. *a*, marks the open efferent end of a single sinus route in early emptying phase. *b*, shows where efferent end of sinus *III* connects to afferent end of sinus *II*. *c*, shows where efferent end of sinus *III a* connects to side of sinus *II*. *d*, shows a venous sinus in conducting phase to distinguish it from pulp partition capillary. *e*, in different positions marks the end of the diagram. At each of these positions the blood vessels would connect to other blood vessels if the diagram were extended. *l*, shows an arterial-capillary entering the side of a venous sinus. *r*, indicates a rare type of connection between sinuses. *cap.*, indicates the long relatively straight capillaries which extend lengthwise through the pulp partitions and end by connecting to venules. Plus signs indicate the positions of the sensitive reactive physiological sphincters whose coordinated actions play so large a part in the control of the circulatory processes of the spleen. The arrows show the direction of blood flow.

In this diagram no effort has been made to show the relative frequency of occurrence of each kind of connection.

course. They pass lengthwise through the pulp partition tissue between the sinuses and connect directly with venules. Many authors have described the first portion of these capillaries, but as far as I know, their ultimate termination has not been previously described. For consideration of function it is to be noted that these long capillaries are connected in shunt with the sinus systems.

The afferent connections of the smallest venules are of three kinds: 1) Efferent ends of single sinus routes, 2) efferent ends of multiple sinus routes, 3) efferent ends of the long, relatively straight capillaries. The smallest venules join together to make larger venules, the structure and connections of which are well known.

Each vessel traced in the living spleen was connected to the arterial system and to the venous system. No vessels have been seen to open out into, or pour blood into intercellular pulp spaces in living unstimulated spleens. No vessels have been seen to end in culs de sac. In the living unstimulated spleen the vascular system consists of a series of completely interconnected, preformed, lined channels.

From the visible structure alone, one might easily conclude that the splenic vascular system is 'closed.' But, that the usual concept of the 'closed' circulation is not adequate to include the functioning of the system becomes quite apparent from a consideration of the cyclic and continuous types of activity of single venous sinuses and multiple sinus routes.

III. Observations of the routine activities of the circulatory systems of spleens that were protected from external stimulation as carefully as possible

A. Control of the distribution of incoming blood. In the spleen there are a number of active, physiological sphincters located at strategic positions on the arterial tree. The sheaths of Schweigger-Seidel (ellipsoids) act as particularly sensitive and powerful sphincters. Müller (1865) traced branches of the post-ganglionic splenic nerves as far as the ellipsoids. It is frequently stated that the diameter of the vessel lumen is

always smaller as it passes through the ellipsoid than at the ellipsoids' ends. This is true only in fixed sections. In the living the diameter varies greatly, depending upon the physiological state of the contractile ellipsoid.

There is also a sphincter on each arterial-capillary which supplies a venous sinus, located a little above the point at which the capillary enters the sinus. And there is one at the efferent end of each sinus.⁴ The sphincters above the afferent end and at the efferent end of the sinuses are as sensitive but not as powerful as those located in the arterial tree. The position of these physiological sphincters is indicated by a plus sign (+) in figure 2. When one of these physiological sphincters is not constricted, the wall at that point shows no indication that it differs from adjacent segments of the vascular system.

In some organs, other than the spleen, the diameters of the terminal arterioles vary from time to time, thus regulating the volume of blood supplied to capillary beds. However, instead of exhibiting only tonus (namely, being partly open or partly constricted) the physiological sphincters exhibit also a valve-like action, changing from fully open to a completely closed condition in a very short time. Not only that, but the timing of the opening and closing of these sphincters seems to indicate that groups of them are controlled, and their activities integrated, by one or more control mechanisms.

The central artery of the malpighian body can contract strongly enough to obliterate its lumen. The sphincters located on each branch of the arterial tree are also capable of

⁴ In connection with the sphincters at the efferent end of the venous sinuses, the question has been raised. "How can there be contraction at this point when no cells have been found here which show the structural characteristics of contractile cells?"

I doubt that we are yet in a position safely to conclude that the only cells which can contract are those showing the same cytological appearance as the units of smooth and striated muscle. Further, the nature of the cells at this point has not been the subject of a specific cytological investigation.

These efferent sphincters have been watched while they contracted sharply, while they opened quickly, and while they remained in a partly closed, namely, in a tonic state. For the description of this, see the sections on the activities of the venous sinuses.

complete contraction. By quickly contracting the sphincters of all but one or two branches of any given stem, all the blood coming in through the stem artery (or arteriole) is directed to one or two open branches. At any given time, blood is directed to small individual areas by being prevented from going anywhere else.

Observations made at the branching of a penicillus in the spleen of mouse series A, no. 1, illustrates the control of the distribution of blood by the integrated action of a set of sphincters. A penicillus having three branches was watched while the animal was completely undisturbed for an hour. A sketch was made and the three branches labeled *a*, *b* and *d*. At any one time, one branch was open and the other two closed. There was a rotation of access to the blood supply. Thus *a* conducted blood, then *d* and then *b*. This order was maintained for 50 minutes in perfect rotation. Each branch was closed twice as long as it was open. One branch opened simultaneously with the closing of another, so that the flow in the stem (that is, in the penicillus) was almost steady and constant. The closed and open phases together in any one branch lasted about 5 minutes.

The control mechanism at this location in this specimen was characterized by integrated mechanical regularity, for 50 minutes, while completely undisturbed. Frequently, however, these sphincter mechanisms exhibit flexibility in responding to minute external applied stimuli, or to unknown stimuli.

Further descriptions of the integrated activity of sets of sphincters will be given later.

The distribution of the blood from the central artery of a malpighian corpuscle to any penicillus, to any branch of a penicillus, to any group of venous sinuses, to any venous sinus and to the red pulp capillaries, is completely and precisely controlled at all times by the coordinated action of these physiological sphincters.

B. The activities of venous sinuses and multiple sinus route systems. Venous sinuses exhibit two types of activity; 'cyclic' and 'continuous.' Both types separate the cells of the blood

from the fluid⁵ of the blood. Sinuses in 'continuous' activity retain the blood cells for a relatively short time. They are retained during long 'storage phases' of the cyclic activity for various periods, from a few minutes up to several hours.

1. The cyclic activity of the venous sinus. The venous sinus has a cycle of activity consisting of, a) a phase of filtration-filling, during which it swells to several times its previous volume, b) a 'storage' phase, c) a phase of sudden emptying, and d) a conducting phase during which whole blood flows into, through, and out of the sinus. Each phase passes smoothly into the next. The cycle is here separated into phases to facilitate description.

a. The filtration-filling phase. At the beginning of the phase of filtration-filling, the sinus appears as a comparatively thick-walled, slightly widened continuation of its own afferent arterial capillary. Its lumen may be three times as wide as that of its afferent vessel, but is usually not more than twice as wide (fig. 3). The flow through the capillary and sinus into the venule at this time is frequently pulsating, similar to that in an arteriole. Suddenly the blood at the efferent end of the sinus backs up a little, as though the sphincter at the efferent end had constricted and obliterated its lumen; and when one focuses on the efferent sphincter it is seen that this has happened (fig. 4). While the efferent sphincter is tightly closed, whole blood continues to come into the sinus from its afferent vessel; blood fluid passes rapidly through the walls of the sinus, blood cells pack into the sinus as the sinus swells and swells, until it becomes a greatly distended cucumber-shaped structure solidly packed with blood cells (fig. 5). At the moment that a sinus is nearly full of packed blood cells, while whole blood is still flowing in, that is, before there is stasis in the afferent vessel, the sphincter located a little above the afferent end of the sinus quickly constricts, suddenly shutting off the blood supply of the sinus before it is quite

⁵ The term 'fluid' is used in this discussion rather than the term 'plasma' because the latter indicates a fluid of known qualitative and quantitative composition. Some of the constituents of plasma may be partly or wholly retained within the sinus, so the term 'blood fluid' is used in order that this question may obviously be left open.

full. I take this as evidence that there is a quantitative control of the volume of blood supplied to a sinus. By this contraction of the sphincter the venous sinus is relieved of the blood pressure of the arterial system while it is packed with blood cells.

In unstimulated spleens red cells have not yet been seen to pass through the lining of the sinus during the filtering-filling phase.



Fig. 3 Conduction phase of the venous sinus cycle.

Fig. 4 Early filtration-filling phase of the venous sinus cycle.

Fig. 5 Late filtration-filling phase of the venous sinus cycle.

Fig. 6 The transition from late filtration-filling to storage phase at the moment the afferent sphincter closes.

Fig. 7 The beginning of the emptying phase when masses of red cells (a part of a soft blood cell 'cast' of the sinus) are being emptied into the venule. *S*, marks the position of sphincters. The arrows show the direction of blood flow.

The separation of cells from fluid is quantitative, for the cells in the sinus are packed so closely together that their individual edges are no longer visible, and, as, the observations of the emptying of the sinus show, there is little fluid in the sinus among the packed blood cells.

While a sinus is in the filtering-filling phase, the finer collecting venules of areas contiguous to that sinus are filled with fluid containing a few red cells which have come from other branches of the venule. It has not yet been possible to discover the exact anatomical position at which blood fluid enters the vascular system again. Because it is transparent, it cannot be seen passing in through the walls of vessels and no holes have been found in the linings of vessels in living unstimulated spleens.

b. The storage phase. The phase of filtering-filling is immediately followed by the 'storage' phase. (This term is used because the retention of blood cells is the visibly prominent feature. However, it is probable that the storage is accompanied by other, much more important processes.) During the storage phase the venous sinus maintains its highly distended condition (fig. 6). This phase usually lasts from a few minutes to 4 hours. However, much longer storage phases have been under continuous observation. In one case (in a young cat) a storage phase lasting for 10 hours was under constant observation. It was followed by several short, apparently normal, complete cycles of activity. This seems to indicate that the tissue was not damaged during the 10-hour storage phase.

During the storage phase while the sinus is highly distended and has a thin wall, some red cells may be seen very slowly passing, individually, from the sinus into the splenic tissue. These red cells are distorted and 'snap' forward as they pass out through the visible membrane-like wall of the sinus.

Specific observation of these red cells in the partitions between the sinuses has thus far failed to show that any of them are phagocytized. In unstimulated spleens the cells of the

pulp partitions are so transparent as to be invisible, but, in watching individual red blood cells in the unstimulated spleen I have not yet seen an erythrocyte lose its outline, change its color, and have its pigment diffuse out around it, in the manner in which these visible evidences of the cytolysis of phagocytized red cells are frequently seen while watching the ingestion and cytolysis of red cells during the agonal changes which occur in this organ.

The number of red cells seen in the pulp partitions adjacent to engorged sinuses in living unstimulated spleens is seldom as large as the number of red cells seen in sections of fixed spleens.

c. The emptying phase. At the end of the storage phase the sphincter at the efferent end of the sinus suddenly opens wide. This opening is almost always startlingly abrupt so that continuous close observation of the sphincter is necessary to see it open (fig. 7). At the moment of its opening irregular masses of blood cells (which are fragments of a soft blood cell 'cast' of the sinus) begin to pass down through the sphincter into the efferent venule. After moving a short distance each mass separates into its individual cells. The blood in the efferent venule of a sinus so discharging is thick and pasty, consisting mostly of cells with very little fluid (fig. 8, V₂ and C). Suddenly the afferent sphincter of the sinus opens, letting whole blood into the afferent end of the sinus. After the entering whole blood washes the packed blood cells out of the sinus, the sinus decreases in diameter until it is but little larger than its afferent capillary. Red cells may be seen at this time slowly passing from adjacent splenic tissue back into the sinus. They 'distort and snap forward' characteristically as they pass through the resistant lining of the sinus.

d. The conduction phase. During the conduction phase, which follows immediately, the flow through the afferent capillary, sinus and even the efferent venule is frequently pulsating as in an arteriole. This is evidence that at this time there is very little resistance to the blood flow through the capillary, sinus and venule. In this phase whole blood flows into the

sinus and whole blood flows out of the sinus. The sinus does not separate blood cells from blood fluid; it conducts whole blood like most other segments of tubing in the vascular system. During the conduction phase there sometimes are periods during which whole blood stands still in the sinus.

At the end of a conducting phase the sinus enters a filtering-filling phase again, and so the cycle repeats. These cycles

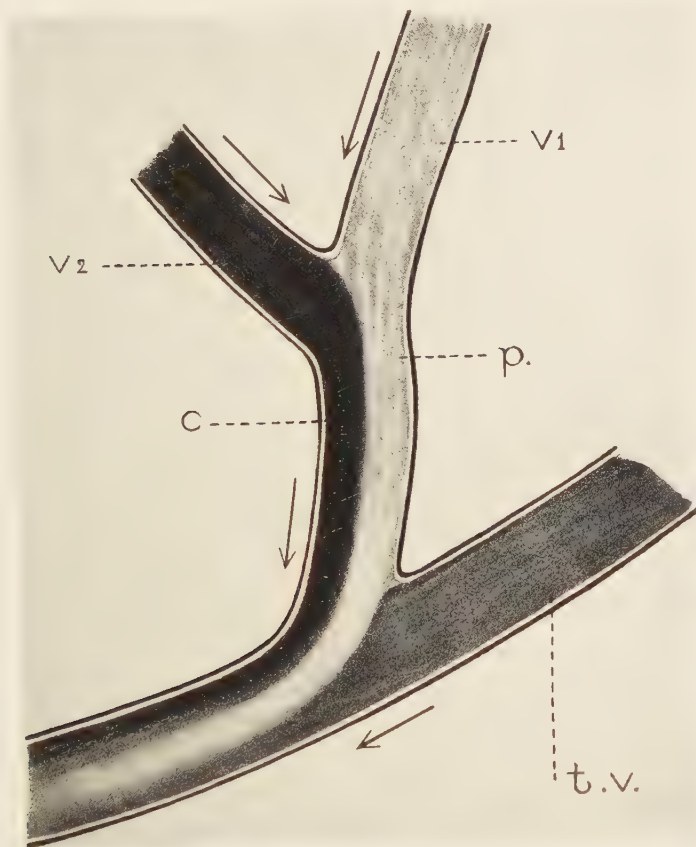


Fig.8 Redrawn from a sketch made while observing an area in spleen of mouse B3. V₁, venule containing almost cell-free blood fluid coming from an area in which the sinuses are retaining the blood cells. V₂, venule containing thick, pasty blood (many cells, little fluid) from an area where sinuses are pouring out blood cell masses. p, fluid, and c cells, which have not yet mixed in the flowing blood. t.v., small trabecular vein. The arrows show the direction of blood flow.

repeat time after time while the spleen is left entirely undisturbed.

2. Structure of venous sinus walls. In fixed sections the individual littoral cells of the sinus wall appear as rods with slits between them (compare Mollier). Careful focusing on living refractile sinus walls shows that in the living organ the entire wall is continuous and is uniform in thickness at any given degree of distension. No slits have yet been seen in the living sinus walls of the three species studied, even under 400 or 600 diameters magnification. Krogh ('29, p. 9) demonstrated the plasticity and elasticity of the frog's red corpuscles. If we can assume that the mammalian red corpuscle is equally plastic and elastic, we would expect that these corpuscles would easily pass through quite small preformed holes in the sinus wall if preformed holes were present. That red cells do not pass consecutively through preformed holes in the walls but pass through the wall by individual penetration, even when the sinus wall is stretched thin in storage phase, indicates that if holes are present they are very small.

During the filtering-filling phase the sinus wall is freely permeable to the colloids of the blood. Blood coming toward a sinus such as the one marked *a* in figure 2 would either pass into the sinus, or into the capillary shunt. If the sinus greatly increased the resistance to flow, the blood would take the alternate route through the capillary. When sinuses are in the filtering-filling phase (and the efferent sphincter closed), one sometimes sees blood flowing freely both into the sinus and through a parallel connected capillary. This is the evidence that at this time the sinus wall is freely permeable to the colloids of the blood. For if the sinus wall were impermeable to colloids (like the membrane of an osmometer) the resistance to the passage of colloids through the sinus wall would very soon create sufficient back pressure so that most of the blood would go via the alternate channel.

When a sinus is in conducting phase, its walls do not permit blood fluid to escape for the cells passing through do not become more and more closely packed as they pass along.

From these facts it is obvious that the sinus wall is capable of considerable variability, the entire range of which probably has not yet been observed.

Sinus walls are probably the only place at which fluid leaves the vascular channels. At least there have been no observations so far to indicate that it leaves the lined system at any place other than the venous sinus walls. In the living spleen no slits have been seen in the lining of the vessels which pass through the ellipsoids, such as are described in sections of fixed spleens by Robinson ('26) and MacNeal ('29). At no point have blood cells been seen coming progressively closer together as they passed along a vessel, as they do in the capillaries of frogs' kidney glomeruli.

3. The integrated cyclic activity of a multiple sinus system. Consider the sinus system consisting of the four sinuses marked I, II, III and III a in figure 2. Sinus I has independent afferent and efferent connections. It can have independent cyclic activity. Sinuses II, III and III a can discharge into the vein only while the efferent sphincter of I is open, that is, when it is in an 'emptying' or 'conducting' phase. Number II does not have an independent arterial-capillary afferent, but III and III a do. Beginning with all four sinuses of the system in conducting phases, the efferents of I, II and III a close. I, II and III a go into filtering-filling phase, followed by storage phase. Whole blood flows through III which remains in conducting phase while II fills. When the afferent sphincter of II closes, III enters its filtering-filling phase and then goes into storage phase. By this time no. I may be emptying, or may already be in conducting phase. Sinus I may go through several independent cycles before II, III and III a discharge. But at some time while no. I is in conducting phase, the efferent of no. II opens wide and it discharges into I, and so out into the venule. Nos. III and III a then discharge through II. All the sinuses of the system are then washed out with arterial blood and the system stays in a common conduction phase for a period. This complex cycle of the multiple sinus system then repeats. The dependent sinuses of a multiple sinus route (as nos. II, III and

III a) differ functionally from independent sinuses (no. I) and single sinus routes, in that they have proportionately longer storage periods. Some multiple sinus systems consist of other numbers of sinuses. Their complex cycles follow the same general principles as that of the four-sinus system described.

As has been said, a sinus system is a definite anatomical unit; its structure and anatomical connections remain constant through all its observed physiological cycles.

4. Continuous separation of cells from fluid, or acyclic function of the venous sinus. Greatly distended single sinuses are seen which have their efferent sphincters partly closed. In these sinuses whole blood flows rapidly and steadily into the afferent end, blood fluid passes rapidly through the sinus wall and thick pasty masses of blood cells continuously squeeze slowly out the efferent end. Meanwhile, clear fluid, with an occasional red cell, is flowing in small adjacent collecting venules.

This type of filtration has not been seen as often as the cyclic functioning of the sinuses. Continuous filtration, when seen, has usually been at a spot, particularly favorable for observation, in the greatly distended spleen of an animal having food in its stomach. It is more difficult to observe the action of sinuses in a spleen in such a distended state than in thinner ones. So it is probable that the rarity of the observation of continuous filtration is not an indication that this activity rarely occurs.

During the continuous filtering process I have not yet seen red cells pass out through the lining of the sinus.

A few times, while a single sinus was being watched, it was observed to change from cyclic activity to continuous filtration and after a time back to cyclic activity again.

5. The time relationships of the phases of venous sinus cycles. The total time occupied by a venous sinus cycle is variable, short under some conditions, long under others. The proportion of the total time of the cycle occupied by a given phase also varies with different conditions. Of the phases,

the emptying phase is usually shortest and least variable. The filtration-filling phase is a little longer and is also more variable. The conduction and storage phases are longest and most variable. The duration of one or both of these two phases occupies most of the time of each cycle.

a. Short cycles. In short (5- or 10-minute) cycles the conduction and storage phases are about equal and together occupy as much as three- or four-fifths of the time of the cycle.

b. During digestion. In early digestion, when an animal has recently ingested food in its stomach and upper small intestine, the sinuses in storage phase are highly distended, and the storage phases are relatively prolonged. On account of the latter, many sinuses are in storage phase simultaneously. At this time some sinuses are distended in continuous filtration periods. The conduction phases are relatively brief. Because so many sinuses are engorged simultaneously, the whole spleen is dilated during the early digestion period.

At this time much of that part of the whole blood which is conducted through the organ without having its cells separated from its fluid goes by way of the long straight capillaries of the pulp partitions, rather than by way of sinuses in conduction phase.

As the time elapses since a meal was ingested, the long storage phases become progressively shorter and the conduction phases longer, so that fewer and fewer sinuses at a given instant are seen distended in storage phase and more and more are seen in conduction phase; each cycle is shorter; and therefore, the spleen becomes progressively smaller and smaller as more time has passed. (However, in the spleen of an animal that has fasted all night, there are still many sinuses in storage phase at a given time.)

c. In fright and exercise. If an animal has been actively exercised and frightened before the spleen is studied, some of the sinuses and sinus systems have relatively brief storage phases, while in other sinus routes arterial blood flows with little resistance in a pulsating stream through arterioles, capillaries and venous sinuses into venules. That is, in an exer-

cised or frightened animal many of the previously stored blood cells are gone, and a great many of the sinuses are in their conducting phases.

d. Reaction to the *local* application of epinephrin. If on the surface of a partly dilated spleen one drops a little epinephrin (a 1/10,000 solution was used, but this was immediately diluted further by the mammalian Ringer's wash solution flowing over the exposed surface of the organ), many sinuses go into emptying phase followed by a period in which arterial blood flows through capillaries, sinuses and sinus systems into venules, in a pulsating stream. A spleen so treated decreases slightly in size; its circulation has somewhat the appearance of that of the spleen of a frightened and exercised animal.

The control mechanisms (namely, sets of sphincters) at the subdivision of the arterioles into arterial-capillaries, regulate the distribution of blood in the venous sinuses and sinus systems, so that the physiological cycles of neighboring sinuses are usually, but not always, out of step with one another. One is filling while another is in storage phase and still a third is emptying, etc. When many adjacent sinuses are seen in conduction, or storage phase at the same time, continuous observation of that area usually shows that one is in the late part of its phase, another in the mid- or early part of its phase, etc.

This control mechanism so operates that the total volume of stored blood cells can be increased or decreased, or, the total can be maintained at a nearly constant level while incoming cells are stored and stored cells are released.

C. The circulation in capillaries. Thus far the connections of and circulation through venous sinuses and multiple sinus systems has been emphasized. It is well to note that the primary function of the venous sinus circulation is not that of nourishing the tissue. It is rather a system which acts upon blood.

The capillary circulation which nourishes the tissue is also important. The capillaries of the malpighian body connect with the fine capillaries that interconnect the branches of the penicilli. The branches of the penicilli have connections with the long straight capillaries which longitudinally traverse the pulp partitions. This capillary network exhibits the irregularly intermittent circulation common to the capillary beds which nourish various tissues. The intermittency of the circulation in the capillaries of the malpighian bodies is roughly similar to the intermittency of the circulation in some of the smaller inguinal lymph nodes of mice. The long straight capillaries of the pulp partitions intermittently conduct blood through the tissue during the storage phases of the venous sinuses. The capillary system is sufficiently independent of the venous sinus system so that regardless of the conditions in the latter the irregularly intermittent nourishing circulation in the former continues.

D. Observations on the circulatory conditions in venules.

At a given time some of the collecting venules, draining a small area containing sinuses, are filled very largely with fluid coming from sinuses in their filtering-filling phase. Other venules are filled with a blood cell paste, containing very little fluid, which is coming from sinuses in their emptying phase. The union of two venules in those two conditions is shown in figure 8. Such a situation has been seen to reverse, that is, a venule having cells later had fluid, and vice versa.

By the time that the cells and fluid reach the larger venules they have been uniformly intermingled. They travel for some distance in this condition and later assume the usual distribution of blood cells and plasma in veins, that is, an axial stream of blood cells in plasma, plus a peripheral layer of plasma.

DISCUSSION

The observations described above show some at least of the routine, physiological activities of the splenic vascular system. Because the animals whose spleens were observed were under an anesthetic—even very lightly under an anesthetic—one

cannot be certain that the processes observed constitute the entire range of the splenic vascular system's capabilities. The rate of the activities described is probably more rapid in the absence of an anesthetic.

No significant differences have yet been observed between the structure and activities of living mouse, rat and cat spleens. No differences have been observed in the circulatory systems of spleens of animals of different ages.

The observations of the spleens of these three species negate the hypothesis that a dilated spleen has an 'open' circulation and a contracted spleen a 'closed' circulation.

Each of the terms 'closed' circulation and 'open' circulation represents the crystallization of ideas around a theory. There was a single primary, unconsciously made, assumption behind each of these two theories. This assumption was: The cells and fluid of blood passing through the spleen travel together, uniformly mixed, just as blood travels through the vascular system of most other organs.

The present observations show that this single primary assumption was erroneous.

Because the sinuses and sinus systems quantitatively separate blood cells from blood fluid, the circulatory systems of the unstimulated spleens of these three species cannot be included under the usual concept of the 'closed' splenic circulatory system. Because whole blood is not freely poured into the intercellular spaces of the pulp partitions the system cannot be included in the usual concepts of the 'open' circulatory system.

On account of their historical associations, neither of the terms 'open' or 'closed' can safely be used to describe the splenic circulation.

Observations, to be reported in the next paper of this series, on the reactions of the splenic vascular system to traumatic stimulation and on the changes which rapidly take place in the spleen while an animal dies, show the probable sources of the great variety of 'open' circulatory system patterns described in fixed spleens.

SUMMARY OF OBSERVATIONS ON UNSTIMULATED SPLEENS

The unstimulated splenic vascular system of mice, rats and cats consists of a system of preformed, interconnected, lined channels. The tissue between the sinuses and sinus systems consists of partitions which appear as 'cords' after cutting the spleen into sections. In the unstimulated spleen few red cells are in these partitions. The few red cells that leave the lined vascular system pass through the lining of the sinus by individual penetration. The sinus lining is resistant enough, even when it is stretched thin in storage phase, to distort the erythrocytes and make them snap forward as they penetrate it. Red cells traveling in the pulp partitions have not yet been seen to be phagocytized or cytolyzed in living spleens.

The venous sinuses quantitatively separate the cells of the blood from the fluid of the blood. There are two ways in which sinuses do this: 1) By the filtering-filling process, and 2) by the continuous filtration process. The sinus wall is the only membrane which has yet been seen to separate blood cells from blood fluid. During the filtration-filling phase venous sinus linings are freely permeable to the colloids of the blood. During the conducting phase blood fluid does not pass out through the sinus wall.

The venous sinuses store blood cells for various periods of time. Under some circumstances incoming blood cells are stored and previously stored cells are released synchronously, so that the total volume of stored cells in a given area of the spleen remains nearly constant. Under other conditions the total volume of stored cells may be increased or decreased. Whole blood can pass through the spleen without having its cells separated from its fluid, by two different routes: 1) By the sinuses and sinus systems when they are in conducting phases, and 2) by way of the long, relatively straight capillaries which pass lengthwise through the partitions between the sinuses. The capillaries conduct blood which nourishes the tissues during the long storage phases of the sinuses.

Precise, integrated control of the distribution of blood is one of the dominant features of the activity of the splenic vascular system. The proportion of blood which passes through the spleen without having its cells separated from its fluid, the proportion having its cells separated from its fluid, and the length of time the cells are stored are continuously controlled. This control is secured by the coordinated action of sets of sensitive, powerful, reactive sphincters located at strategic positions. The sheaths of Schweigger-Seidel act as especially powerful sphincters.

The venous sinuses and venous sinus systems are not primarily a system of vessels which conduct blood to nourish the splenic tissue. The sinus systems and pulp partitions constitute, rather, a mechanism which acts upon blood.

On account of their historical associations, and the limited concepts they indicate, neither of the terms 'closed' or 'open' can safely be used to designate the splenic circulation.

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